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Carbon Disulfide: A Semiochemical Mediating Socially-Induced Diet Choice in Rats

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GALEF, B. G., JR., J. R. MASON, G. PRETI AND N. J. BEAN. *Carbon disulfide: A semiochemical mediating socially-induced diet choice in rats.* PHYSIOL BEHAV **42**(2) 119–124, 1988.—Gas chromatography/mass spectrometry revealed the presence of both carbon disulfide (CS_2) and carbonyl sulfide (COS) on rat breath. Behavioral experiments indicated that rats exposed to an unfamiliar diet moistened with CS_2, like rats exposed to an unfamilar diet placed on the fur of an anesthetized rat, subsequently exhibited enhanced preference for the unfamiliar diet. Rats in experimental groups: (a) interacted for 30 min with a wad of cotton batting powdered with one of two unfamiliar foods (either Diet A or Diet B) and moistened with a dilute, aqueous CS_2 solution, (b) ate Diets A and B in succession and finally, (c) were injected with LiCl. In a subsequent choice between Diets A and B, these rats exhibited a preference for whichever of the foods had been present on the cotton batting during (a). Rats in control groups were treated identically to those in experimental groups, except that the diet-coated cotton batting to which they were exposed was moistened with distilled water rather then CS_2 solution. Rats in control groups were not affected in their later diet choice by the food present on the cotton batting during (a). These data are consistent with the hypothesis that CS_2 is a semiochemical that mediates social influence on diet selection in rats.

Semiochemical Social transmission Diet selection Food preference Social learning

RESULTS of a number of recent studies indicate that rats exhibit enhanced preference for a food after interacting with conspecifics that have eaten that food [12,14]. If offered a choice between two unfamiliar diets (Diets A and B), naive "observer" rats that had previously interacted with "demonstrator" rats fed Diet A prefered Diet A, while naive observer rats that had interacted with demonstrator rats fed Diet B, preferred that diet.

In the situations studied by Galef and his coworkers, influence of demonstrators on the later diet preferences of their adult observers was not the result of simple exposure of observers to the odor (or taste) of the diets that their respective demonstrators had eaten ([9–11]; but see [14]). Post-weaning domesticated rats did not prefer a food after they had eaten or smelled it; they did prefer the same food after they had encountered it on the fur of a demonstrator rat [9–11].

Even experience of a food in association with a demonstrator rat was not always sufficient to produce subsequent enhancement of preference for that food. Preference enhancement depended upon details of the context in which exposure to a diet occurred [10,11]. For example, naive rats that interacted with either (a) the head end of a demonstrator rat recently sacrificed by anesthetic overdose and powdered with food or (b) the hind end of an anesthetized demonstrator rat powdered with food did not develop a preference for the

food placed on their respective demonstrators. On the other hand, rats interacting with the front end of an anesthetized demonstrator rat powdered with a food developed a preference for the food with which their respective demonstrator rats were powdered [10,11].

One obvious difference between both (a) the front ends of live and dead rats and (b) the front and hind ends of live rats is that the former member of each pair (effective as a context for enhancing diet preference) expels breath, while the latter member of each pair (relatively ineffective as a context for enhancing diet preference) does not. In sum, simultaneous exposure to a food and to rat breath has resulted in subsequent enhanced preference for the food, while exposure to a food in the absence of rat breath has not resulted in enhanced preference for the food. In the experiments reported below, we determined whether exposure to a chemically-identified component of rat breath, in combination with exposure to a food, might cause changes in subsequent response to the food similar to those caused by simultaneous exposure to a food and a breathing conspecific.

EXPERIMENT 1

The first problem encountered in attempting to identify components of rat breath that might support social influence on diet selection is to exclude from consideration general, rat-produced volatiles that are not very effective in altering responses to foods. For example, as mentioned above, the rear of a rat, though presumably smelling ratlike, does not provide a very effective context for altering diet preference in other rats [11].

Rats are an excellent species for differentiating general, species-typical volatiles from volatiles specific to breath. Rats breathe only through their noses, not through their mouths. Hence, if volatiles providing an effective context for altering response to a diet are not simply general rat odors, but are to be found only in rat breath, comparison of the chemical constitutents of air removed from the noses and mouths of rats should permit the identification of potentially effective volatiles. Volatile, sulfur-containing compounds are known to be detectable even at concentrations of a few parts/billion [13]. Further, volatile sulfur compounds have been implicated as semiochemicals in several mammalian species [1, 2, 15]. Consequently, we targeted our analyses of samples of air taken from the noses and mouths of anesthetized rats on sulfur compounds.

Subjects

Three Sprague-Dawley and two Long-Evans, 75-day-old, female rats obtained from Charles River Breeding Laboratories served as subjects in the present experiment. All subjects were maintained in individual cages on ad lib Purina Laboratory Rodent Chow and water prior to and during the experiment. Each subject was anesthetized by IP injection of sodium pentobarbital (50 mg/kg) immediately prior to its participation in the experiment. Each subject served three times, at weekly intervals.

Procedure

Sampling. Samples of air were collected from both the nasal and oral cavities of subjects using gas-tight, 30-ml, disposable syringes. To take a sample from the oral or nasal cavity of a rat, one end of a length of 6.3 mm (i.d.) Tygon tubing was slipped over the end of a syringe and the other end of the tubing was held over the nose or in the mouth of an anesthetized rat while the syringe was filled with air at a rate of approximately 1 cc/sec.

To assure that sufficient quantities of volatiles were available for chemical identification, 25 alliquots were drawn from subjects of the same strain and injected into 1-liter Teflon bags, creating a single pooled sample of air from either the oral or nasal cavities of two or three subjects. Alternatively, samples of air taken from the noses or mouths of two or three subjects of the same strain were injected directly into tubes containing absorbant until a pooled sample of 180 ml of air had been collected. In all, six pooled samples of air from the nasal cavities and six pooled samples of air from the oral cavities of rats of two strains were examined chemically.

Analysis. Initially, Tenax (Suppelco, Inc., State College, PA) was employed to trap volatiles. Analysis of the volatiles collected using Tenax indicated the presence of sulfur compounds. Because Tenax is not an ideal absorbent for such compounds, subsequent collections and analyses employed a "Custom Absorbent for Sulfur Gases" (a bonded polymer available from Suppelco Inc., State College, PA).

For desorption of collected volatiles, the absorbent tubes were placed in a semiautomated tube desorber (Envirochem, Inc., Kembelsville, PA). Volatiles were desorbed over a 3-min period by rapid heating to 250°C in a helium stream. After desorption, the volatiles were condensed onto the first 15–20 cm of a capillary column that was cooled by nitrogen circulating through a copper coil immersed in a liquid nitrogen bath.

Analyses of desorbed volatiles was performed using a Finnegan/MAT 4510 GC/MS data system equipped with a split/splitless injector, a fused silica capillary column, and the capability of operating in both electron impact and chemical ionization modes. The column employed for chromatography contained a bonded Carbowax-like phase (Stabilwax, 30 M × 0.32 mm) with a 0.25 micron coating (Restek Corp., Port Matilda, PA). The chromatograph was programmed from 60° (8-min hold) to 100°C at 3°C/min. The spectrometer was interfaced with a Nova 4X computer which utilized Super Incos(R) software for data acquisition and analysis. The mass range of m/z 40–350 was scanned once each sec, and the typical run included 1500 1-sec scans. Acquisition of mass spectral data was begun the moment heat was applied to the tubes. This insured that even if an organic compound of high volatility leaked through the cold trap, its mass spectrum would be obtained.

Identification of volatiles in nasal and mouth air samples was based on interpretation of the spectra by one of us (GP) as well as comparison of the mass spectra with both: (a) mass spectra contained in the National Bureau of Standards (NBS) library of 31,000 compounds and (b) mass spectra obtained from commercially available standards. The latter standards were also used to compare chromatographic retention times with COS and CS_2 in rodent breath. The computer reconstructed ion chromatograms were searched for ions (via mass chromatograms) and/or spectra characteristic of volatile sulfur-containing compounds [e.g., hydrogen sulfide, methylmercaptane, dimethylsulfide, carbonyl sulfide (COS), carbon disulfide (CS_2)].

Results

No sulfur containing volatiles were detected in any of the air samples taken from the oral cavities of our subjects. Conversely, CS_2 and COS were both detected in all air samples taken from the nasal cavities of subjects. No evidence of other sulfur volatiles was obtained.

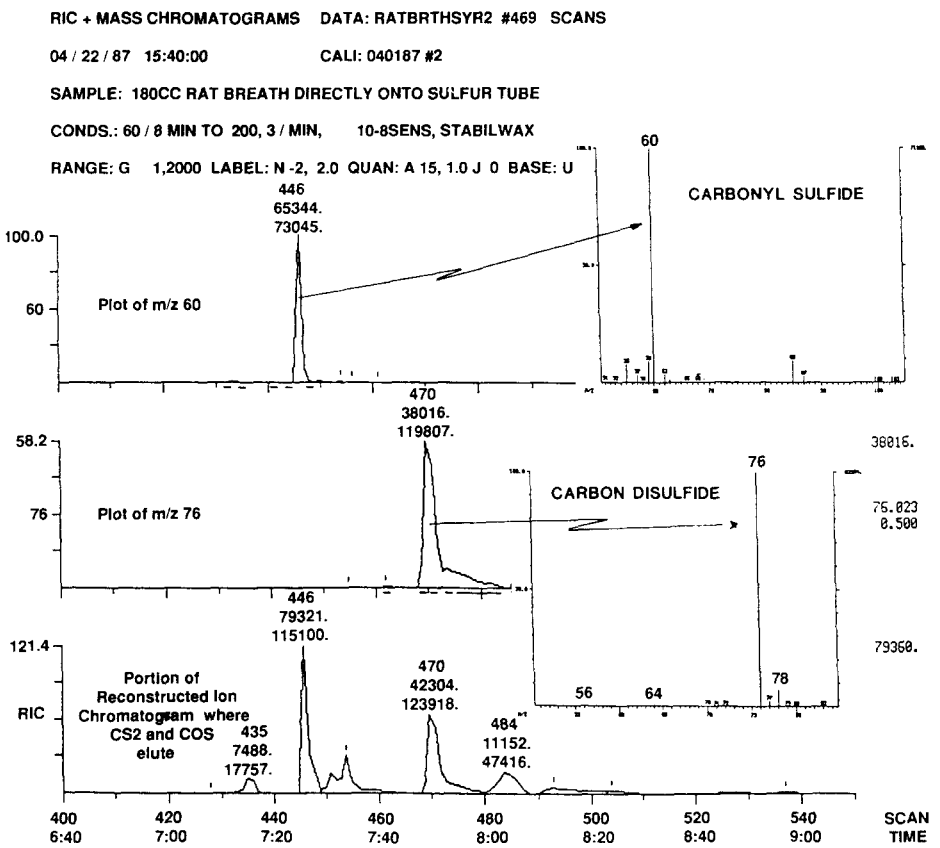

FIG. 1. Reconstructed ion chromatograms of rat breath showing molecular ions for COS and CS_2. The spectrum of CS_2 consists mainly of the molecular ion m/z 76, the sulfur-containing isotope ion at m/z 78 as well as a small fragment ion at m/z 64. The spectrum of COS consists primarily of its molecular ion at m/z 60, and an isotope ion at m/z 62.

A typical reconstructed ion chromatogram generated by the data system, as well as the points at which COS and CS_2 eluted are shown in Fig. 1. The elution of COS is indicated by the maximum in the mass chromatogram of m/z 60 (molecular ion of COS), while the elution of CS_2 is indicated by the maximum in the mass chromatogram of m/z 76 (the molecular ion of CS_2). The spectra for these compounds have been inserted into the figure; both are extremely simple. The spectrum for CS_2 consists mainly of the molecular ion m/z 76, the sulfur-containing isotope ion at m/z 78, as well as a small fragment ion at m/z 64. The spectrum of COS consists primarily of its molecular ion at m/z 60, and an isotope ion at m/z 62.

Subsequent experiments employing procedures identical to those described above with standard amounts of CS_2 (Fisher Scientific) and COS (Thermedics, Woburn, MA) permitted calculations of the CS_2 concentrations (about 1-ppm) on breath, as well as retention times for the compounds. These are 413 sec and 433 sec for COS and CS_2, respectively.

EXPERIMENT 2

The finding that carbon disulfide (CS_2) and carbonyl sulfide (COS) are present in air taken from the noses, but not the mouths, of rats is, obviously, not in itself evidence that either compound is a semiochemical active in social influence on diet selection. Because COS is a gas at room temperature, we have not been able to examine effects of COS on food preference. We have reported elsewhere [3] that the presence of CS_2 increases attractiveness of a food to mice. However, the finding that a dilute solution of CS_2 increases the attractiveness of a food to which it is added is not really relevant to the question of whether CS_2 plays a role in social transmission of diet preference. In cases of social transmission of diet preference [4–12], exposure to a food in an appropriate social context increases preference for the food when it is later presented without the social context.

In the most reliable of several previously-described procedures demonstrating social influence on diet choice [4–12], a demonstrator rat was fed one of two diets (either Diet A or Diet B) and then allowed to interact with a naive observer. The observer was next fed both Diets A and B in rapid succession and poisoned by IP injection of LiCl. Following recovery from toxicosis, the observer was offered a choice between Diets A and B. Under such conditions, observers repeatedly showed a robust preference for whichever diet (A or B) their respective demonstrators had eaten [6,8]. These socially-induced preferences have been interpreted [6,8] as providing evidence of a role of social influence in poison avoidance learning [4,7], but, for purposes of the present paper, they need only be treated as reliably demonstrating social influence on subsequent diet choice in rats.

As mentioned in the introduction to the present paper, it has been shown [9–11] that those Norway rats exposed to a

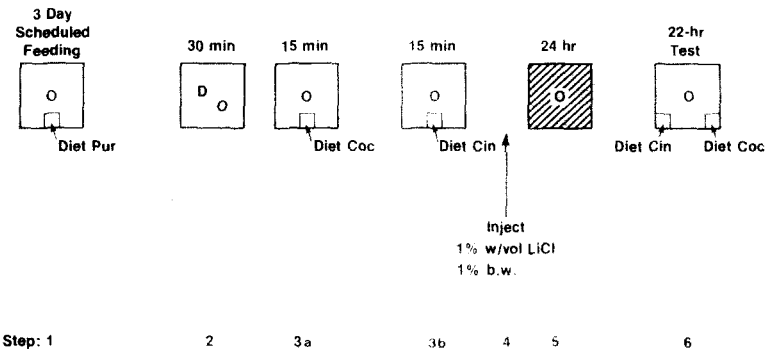

FIG. 2. Schematic of the procedure of Experiment 2. Diet Pur = Powdered Purina Laboratory Rodent Chow. Diagonal striping = ad lib access to pellets of Purina Laboratory Rodent Chow.

food on an anesthetized conspecific, but not those rats simply exposed to the food alone, subsequently exhibit an enhanced preference for the food. If we were to find that CS_2 acted, as does the presence of a conspecific, to provide a context within which exposure to a food enhanced subsequent preference for that food, such evidence would support the hypothesis that CS_2 plays a role in normally-occurring social transmission of diet choice in rats [4–12].

Subjects

Seventy-two, 42-day-old, female, Long-Evans rats, purchased from Charles River Canada (St. Constant, Quebec), served as observers in the present experiment. An additional 16, 56-day-old, female, Long-Evans rats that had served as observers in earlier experiments served as demonstrators. All subjects were maintained on ad lib Purina Laboratory Rodent Chow and water in temperature and humidity controlled colony rooms on a 12:12 light:dark schedule.

Apparatus

Observers and demonstrators were housed individually throughout the experiment, the former in 22×24×27.5 cm wire-mesh hanging cages, the latter in plastic shoe-box cages kept in a room separate from the observers. During the period of interaction of demonstrators and observers (Step 3 of Procedure, see Fig. 1), each observer and its demonstrator were placed in an apparatus (illustrated in Fig. 4 of [10] and [11]) constructed from a 2.45-l (15.2 cm high, 19.0 cm top-diameter, 14.0 cm bottom-diameter) cardboard bucket (Lily-Tulip Inc., Toledo, OH) of the type commonly used by fast-food franchises.

A .63-cm (1/4-in), hardware-cloth tube, 16 cm long × 5 cm dia. was inserted for half its length through a 5-cm dia. circular opening cut in the wall of the bucket, 12 cm above the bucket floor. The end of the tube inside the bucket was closed with hardware cloth; the end outside the bucket was left open. A cardboard lid was used to prevent observers from leaving the bucket.

Surrogate Rats

Surrogate rats were made by wrapping rat-sized pieces of cotton-batting in surgical gauze tubing and rolling one end of the resulting cylindrical surrogates in a diet. The diet-coated end of a surrogate was then placed in the same location in the hardware-cloth tube that the head of a demonstrator rat would have occupied if it were placed in the hardware-cloth tube.

Procedure

Treatment of observers (n=48) and demonstrators (n=16) during the experiment was as follows (see Fig. 2).

Step 1. Observers were introduced into their cages and placed on a 23-hr food deprivation schedule, receiving powdered Purina Laboratory Rodent Chow for 1 hr/day for 2 days.

Step 2. Following a third 23-hr period of food deprivation, each observer was removed from its cage and placed for 1/2 hr in the bucket of an apparatus like that illustrated in Fig. 1. The wire-mesh tube of the apparatus contained one of six different kinds of demonstrator, depending on the group to which an observer had been randomly assigned.

Observers assigned to either Cin-Anes-Dem (n=8) or Coc-Anes-Dem (n=8) Groups were placed in buckets to interact with a demonstrator anesthetized by intraperitoneal injection of 50 mg/kg of sodium pentobarbital. The face of each anesthetized demonstrator had been rolled either in Diet Cin (powdered Purina Laboratory Rodent Chow adulterated 1% by weight with McCormick's pure ground cinnamon) or in Diet Coc (powdered Purina Laboratory Rodent Chow adulterated 2% by weight with Hershey's Cocoa). Each observer assigned to either Coc-Surr + CS_2 (n=8) or Cin-Surr + CS_2 (n=8) Groups interacted for 1/2 hr with a surrogate demonstrator one end of which had been rolled either in Diet Coc or in Diet Cin and then moistened with six drops of a 1 ppm solution of CS_2 in distilled water. Those observers assigned to either Coc-Surr (n=8) or Cin-Surr (n=8) Groups interacted for 1/2 hr with a surrogate rolled either in Diet Cin or in Diet Coc and moistened with six drops of distilled water.

Steps 3a and 3b. At the end of the 1/2-hr period of interaction, each observer was returned to its cage and offered a weighed food cup containing Diet Coc. This food cup was left in the observer's cage for 15 min. At the end of this first 15-min observer feeding period, the food cup containing Diet Coc was removed and replaced for 15 min with a second, weighed food cup containing Diet Cin.

Step 4. Immediately following termination of the second feeding period, each observer was injected IP with 1% of body weight, 1% w/v LiCl solution.

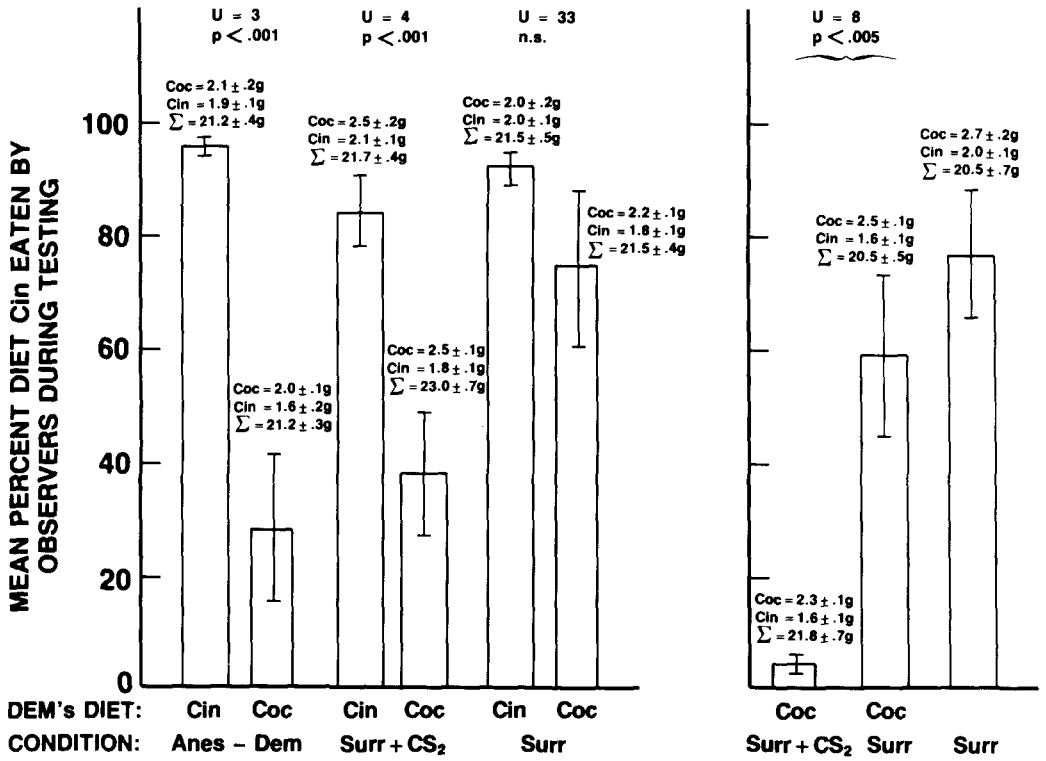

FIG. 3. Mean amount of Diet Cin eaten, as a percentage of total amount ingested by observers in the various groups during testing (Step 6 of Procedure). Means and SEMs above histograms: Coc = Mean g Diet Coc eaten during Step 3 of Procedure; Cin = Mean g Diet Cin eaten during Step 3 of Procedure; Σ = Mean total g eaten during Step 6 of Procedure.

Step 5. One hour following injection, pellets of Purina Laboratory Rodent Chow were placed in each observer's cage, and each was given 24 hr to recover from the effects of toxicosis.

Step 6. Following the 24-hr recovery period, each observer was offered, for 22 hr, a simultaneous choice of weighed samples of Diet Cin and Diet Coc.

At the end of the 22-hr test period, the experimenter determined each observer's intake of Diet Cin and Diet Coc and calculated the percentage of Diet Cin eaten by each observer.

Results and Discussion

The main results of Experiment 2 are presented in the left-hand panel of Fig. 3, which shows the mean amount of Diet Cin eaten by observers during testing (Step 6 of Procedure), as a percentage of the total amount the observers ate during the 22-hr test session.

As can be seen from examination of the left-hand panel of Fig. 3, observers in Anes-Dem and Surr + CS_2 Groups exhibited a significant tendency to ingest preferentially the diet placed on their respective demonstrators or surrogates (Mann-Whitney U-tests, both Us<4, both $ps<0.001$). To the contrary, observers in Surr Groups did not show a tendency to ingest the diet to which they were exposed for 1/2 hr during Step 2 of the procedure (U=33, p=n.s.).

The results of Experiment 2 indicate that observers that experienced a diet in association with CS_2, like observers that experienced a diet on the head of a demonstrator, subsequently exhibited a significant preference for that diet. Observers simply experiencing a diet without simultaneous exposure to CS_2 did not exhibit a preference for it.

EXPERIMENT 3

Upon completion of Experiment 2, we identified the most informative groups in that experiment and conducted a partial replicate and extension of it.

Subjects

Twenty-four experimentally naive 42-day-old, female Long-Evans rats served as observers in the present experiment.

Procedure

The apparatus and methods of Experiment 3 were identical to those of Experiment 2. In the present experiment, eight observers interacted for 1/2-hr during Step 2 with either: (a) a surrogate rolled in Diet Coc and moistened with 6 drops of CS_2 at a concentration of 1 ppm (Coc-Surr + CS_2 Group), (b) a surrogate rolled in Diet Coc and moistened with six drops of distilled water (Coc-Surr Group) or (c) a surrogate neither rolled in diet nor moistened with liquid (Surr Group).

Results and Discussion

The main results of Experiment 3 are presented in the right-hand panel of Fig. 3. Examination of the panel reveals that observers in the Coc-Surr + CS_2 Group ate a smaller

percentage of Diet Cin (and, therefore, a greater percentage of Diet Coc) than did those observers in the Coc-Surr Group. These data replicate the main result of Experiment 2. Comparison of the diet choices of subjects in the Coc-Surr and Surr Groups revealed no difference in their diet preferences.

Exposure to a surrogate dusted with Diet Coc had no effect on subjects' subsequent choice between Diets Cin and Coc. Exposure to similar surrogates dusted with Diet Coc and moistened with CS_2 solution profoundly affected subjects' subsequent choice between Diets Cin and Coc. We conclude that CS_2 can act, as does the presence of a demonstrator rat, as a context that renders exposure to a diet effective in influencing an observer's later preference for that diet.

GENERAL DISCUSSION

The results of Experiments 2 and 3 are consistent with the outcome of previous studies [9–11] indicating that enhanced preference for a diet after experience of the diet in association with a conspecific demonstrator is not the result of simple exposure to the diet. In Experiments 2 and 3 of the present series, as in previous experiments, preference enhancement depended on exposure to a diet within a context provided either by a rat or by CS_2. Observers in Experiments 2 and 3, interacting either with anesthetized demonstrators powdered with a diet or with CS_2-moistened surrogates, subsequently preferred that diet; observers interacting with surrogates simply powdered with a diet were not affected in their subsequent diet choice by the experience.

The results of Experiment 1 indicate that carbon disulfide (CS_2) is carried on the breath of rats. The main results of Experiments 2 and 3 indicate that the presence of CS_2, like the presence of an anesthetized demonstrator rat, rendered exposure to a diet effective in enhancing later preference for that diet. Taken together, the results of the present studies suggest that CS_2 may provide an important contextual component for the social transmission of food preferences among rats.

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